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The dipeptidyl peptidase IV inhibitors vildagliptin and K-579 inhibit a phospholipase C: a case of promiscuous scaffolds in proteins.

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Authors

Chakraborty, Sandeep
Rendón-Ramírez, Adela
Ásgeirsson, Bjarni
et al.

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The dipeptidyl peptidase IV inhibitors vildagliptin and K-579 inhibit a phospholipase C: a case of promiscuous scaffolds in proteins [v3; ref status: indexed, <http://f1000r.es/51m>]

Sandeep Chakraborty^{1,2*}, Adela Rendón-Ramírez^{3*}, Bjarni Ásgeirsson^{4*},
Mouparna Dutta⁵, Anindya S. Ghosh⁵, Masataka Oda⁶, Ravindra Venkatramani⁷,
Basuthkar J. Rao¹, Abhaya M. Dandekar², Félix M. Goñi³

¹Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, 400 005, India

²Plant Sciences Department, University of California, Davis, CA, 95616, USA

³Unidad de Bio, Universidad del Pais Vasco, Bilbao, Spain

⁴Science Institute, Department of Biochemistry, University of Iceland, IS-107 Reykjavik, Iceland

⁵Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur, 721302, India

⁶Division of Microbiology and Infectious Diseases, Niigata University Graduate School of Medical and Dental Sciences, Niigata, 951-8514, Japan

⁷Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai, 400 005, India

* Equal contributors

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Abstract

The long term side effects of any newly introduced drug is a subject of intense research, and often raging controversies. One such example is the dipeptidyl peptidase-IV (DPP4) inhibitor used for treating type 2 diabetes, which is inconclusively implicated in increased susceptibility to acute pancreatitis. Previously, based on a computational analysis of the spatial and electrostatic properties of active site residues, we have demonstrated that phosphoinositide-specific phospholipase C (PI-PLC) from *Bacillus cereus* is a prolyl peptidase using *in vivo* experiments. In the current work, we first report the inhibition of the native activity of PI-PLC by two DPP4 inhibitors - vildagliptin (LAF-237) and K-579. While vildagliptin inhibited PI-PLC at micromolar concentrations, K-579 was a potent inhibitor even at nanomolar concentrations. Subsequently, we queried a comprehensive, non-redundant set of 5000 human proteins (50% similarity cutoff) with known structures using serine protease (SPASE) motifs derived from trypsin and DPP4. A pancreatic lipase and a gastric lipase are among the proteins that are identified as proteins having promiscuous SPASE scaffolds that could interact with DPP4 inhibitors. The presence of such scaffolds in human lipases is expected since they share the same catalytic mechanism with PI-PLC. However our methodology also detects other proteins, often with a completely different enzymatic mechanism, that have significantly congruent domains with the SPASE motifs. The reported elevated levels of serum lipase, although contested, could be rationalized by

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report



report



report

1 **Rodney Rouse**, U.S. Food and Drug
Administration USA

inhibition of lipases reported here. In an effort to further our understanding of the spatial and electrostatic basis of DPP4 inhibitors, we have also done a comprehensive analysis of all 76 known DPP4 structures liganded to inhibitors till date. Also, the methodology presented here can be easily adopted for other drugs, and provide the first line of filtering in the identification of pathways that might be inadvertently affected due to promiscuous scaffolds in proteins.

2 Mark D Gorrell, Centenary Institute
Australia

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Corresponding author: Sandeep Chakraborty (sanchak@gmail.com)

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REVISED Amendments from Version 2

In the current version, we have changed the title, and cited previous research (ref 41 and 54) based on referee suggestions.

We have also included some minor corrections as suggested by a co-author.

See referee reports

Introduction

Oral glucose elicits a greater insulin response than intravenous glucose infusion, a phenomenon known as the incretin effect¹. This effect is mostly attributed to the intestinally derived hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP)². These hormones have a very short half-life as they are rapidly inactivated by the ubiquitous enzyme dipeptidyl peptidase-IV (DPP4)³. The finding that the incretin effect is impaired in subjects with type 2 diabetes⁴ led to two major types of GLP-1 based therapies⁵ - intravenously or sub-cutaneously administered GLP-1 mimetics that are resistant to DPP4 (exenatide, liraglutide, etc.)⁶, and the orally administered gliptins that prolong the physiological actions of incretin hormones by inhibiting DPP4 (sitagliptin, vildagliptin, etc.)⁷⁻⁹. Due to the multifarious roles played by the DPP4 enzyme¹⁰⁻¹², the possible side effects of these drugs (acute pancreatitis, pancreatic cancer, etc.¹³⁻¹⁵) are strongly contested by researchers who argue that current statistics are insufficient^{16,17} to conclusively attribute these side effects to the otherwise beneficial GLP-1 drugs¹⁸. Compound promiscuity is another phenomenon that might play a crucial role in determining the side effects of these therapies, although this aspect has rarely been pursued intensively¹⁹.

Previous work by our group has established the spatial and electrostatic congruence in cognate residue pairs of the active site in proteins with the same functionality (CLASP)^{20,21}. CLASP analysis indicated that the phosphoinositide-specific phospholipase C (PI-PLC) from *Bacillus cereus* has spatial and electrostatic congruence with a serine protease motif²². This was validated by protease assays, mass spectrometry and by inhibition of the native phospholipase activity of PI-PLC by the well-known serine protease inhibitor AEBSF ($IC_{50} = 0.018$ mM). The specificity of the protease activity was for a proline in the amino terminal, suggesting that PI-PLC is a prolyl peptidase, similar to the DPP4 enzyme. This finding led us to believe that the gliptins would have similar inhibitory effect on PI-PLC. In the current work, we have confirmed the inhibition of the native phospholipase activity of PI-PLC using two gliptins - vildagliptin²³ (at μ -molar concentrations) and K579²⁴ (at nano-molar concentrations).

Subsequently, we used a motif derived from a DPP4 protein²⁵, in addition to the trypsin motif used previously²², to query a comprehensive and non-redundant (50% sequence identity) list of ~5000 human proteins with known structures using CLASP, intending to identify other proteins that might be inhibited by the gliptins. From the set of proteins with significant congruent matches with these two motifs, we identified a pancreatic lipase²⁶ and a gastric lipase²⁷,

keeping the context of lipases, acute pancreatitis and GLP-1 based therapies in mind. Our findings rationalize the elevated levels of serum lipase found in patients undergoing DPP4 inhibitor based therapies^{28,29}, although these reports are in disagreement with other findings^{30,31}. While it is logical and expected to find scaffolds that are congruent to trypsin and DPP4 active sites in lipases based on the current results and our previous findings²², we also show the presence of the serine catalytic triad in close proximity to the active site residues of proteins which have a completely different enzymatic mechanism (for example, in glutaminyl cyclase which is a transferase³²). This corroborates the current belief that convergent evolution occurs more frequently than previously believed³³. Thus, we propose a rational method to identify proteins that might have unintended and undesirable interactions with newly introduced compounds, and substantiate our claims by demonstrating the inhibition of the native phospholipase activity of PI-PLC from *B. cereus* using gliptins that are used in type 2 diabetes therapy.

Results

The active site motifs

The active sites of serine proteases differ in their specificities owing to residues other than the conserved catalytic triad. Thus, in addition to the trypsin motif used previously (Asp102, Ser195 and His57 - PDBid 1A0J)²² (Motif1), we choose another motif from a DPP4 enzyme (Asp708, Ser630 and His740 - PDBid:1N1M) (Motif2) (Table 1). Apart from the catalytic triad, we chose another non-polar residue in order to increase the specificity of the matches (Ala56 in Motif1 and Val711 in Motif2). This fourth residue is chosen as the closest residue to any one of the catalytic triad residues. Using the ability of CLASP to include stereochemically equivalent residues, this last residue could be matched by another non-polar residue - one of Gly, Ala, Val, Leu, Ile or Met. Further, it has been seen that the second (ac) and fifth (bd) (Table 1) pairwise electrostatic potential differences (EPD) are not discriminatory - thus, this pair is not used to score the EPD difference (although it is included in the distance deviation score).

Inhibition of phosphoinositide-specific phospholipase C (PI-PLC) using dipeptidyl peptidase-IV (DPP4) inhibitors. DPP4 (EC 3.4.14.5), a serine protease that is expressed in many tissues (kidney, liver, lung, intestinal membranes, lymphocytes and endothelial cells), cleaves peptides with Pro or Ala residues in the second amino terminal position. Previously, we have experimentally demonstrated the existence of the serine protease domain in PI-PLC from *Bacillus cereus* - both by virtue of its proteolytic activity, and the inhibition of its native activity on phospholipids in the presence of serine protease inhibitors²². Furthermore, the specificity of the proteolytic activity indicated that it was a prolyl peptidase - thus, leading us to believe that DPP4 inhibitors should have a similar inhibitory effect on the PI-PLC enzyme. Table 1 shows the presence of a congruent motif in the PI-PLC protein with both Motif1 and Motif2. His32 and Asp67 are known to be a part of the active site scaffold in PI-PLC²². These proteins have completely different folds, and thus a superimposition (using both MUSTANG³⁴ and DECAAF³⁵) does not show any detectable similarity in their structures (Supplementary Figure 1). Figure 1 shows the active sites of these proteins, and the superimposition of these proteins

Table 1. Potential and spatial congruence of the active site residues in proteins queried using two motifs - Motif1 from Trypsin and Motif2 from DPP4. Rmsd1 and Rmsd2 are the root mean square deviation of the scaffold with respect to Motif1 and Motif2. DPP4 - dipeptidyl peptidase-IV, PI-PLC - phosphoinositide-specific phospholipase C, PLASE - human pancreatic lipase-Related Protein 2, GPASE - human gastric lipase, QC - glutaminyl cyclase. D = Pairwise distance in Å. PD = Pairwise potential difference. APBS writes out the electrostatic potential in dimensionless units of kT/e where k is Boltzmann's constant, T is the temperature in K and e is the charge of an electron.

| PDB | Active site atoms (a,b,c,d) | | ab | ac | ad | bc | bd | cd | Rmsd1 | Rmsd2 |
|------------------------|-----------------------------|---------|---------------|--------------|---------------|--------------|----------------|---------------|-------|-------|
| TRYPSIN (1A0J) | D102,S195 H57,A56 | D PD | 7.8 -144.1 | 5.6 -39.2 | 2.9 -248.3 | 3.3 104.8 | 9.0 -104.3 | 6.9 -209.1 | 0 | 0.5 |
| DPP4 (1N1M) | D708,S630 H740,V711 | D PD | 7.6 -154.4 | 5.4 124.4 | 2.6 -148.8 | 2.6 278.8 | 6.8 5.6 | 5.4 -273.2 | 0.5 | 0 |
| PI-PLC (1PTD) | D67,S234 H32,I68 | D PD | 8.2 -93.7 | 6.2 39.7 | 4.1 -245.2 | 3.8 133.4 | 11.5 -151.5 | 9.2 -284.8 | 0.6 | 1.1 |
| PLASE (2OXE) | D195,S171 H282,G235 | D PD | 7.7 -150.2 | 6.4 26.7 | 4.4 -132.1 | 3.0 176.9 | 6.7 18.2 | 5.8 -158.8 | 0.5 | 0.4 |
| GPASE (1HLG) Motif1 | D324,S153, H353,L326 | D PD | 7.5 -202.6 | 5.0 -15.0 | 2.9 -272.3 | 2.7 187.6 | 8.4 -69.7 | 6.2 -257.3 | 0.2 | 0.3 |
| GPASE (1HLG) Motif2 | D324,S153 H353,A327 | D PD | 7.5 -202.6 | 5.0 -15.0 | 2.6 -207.1 | 2.7 187.6 | 7.1 -4.5 | 5.3 -192.1 | 0.4 | 0.1 |
| QC (3PB4) | D170,S187, H168,G224 | D PD | 7.5 -92.8 | 4.8 -16.5 | 3.4 -214.0 | 3.3 76.3 | 10.7 -121.2 | 8.0 -197.5 | 0.4 | 0.8 |

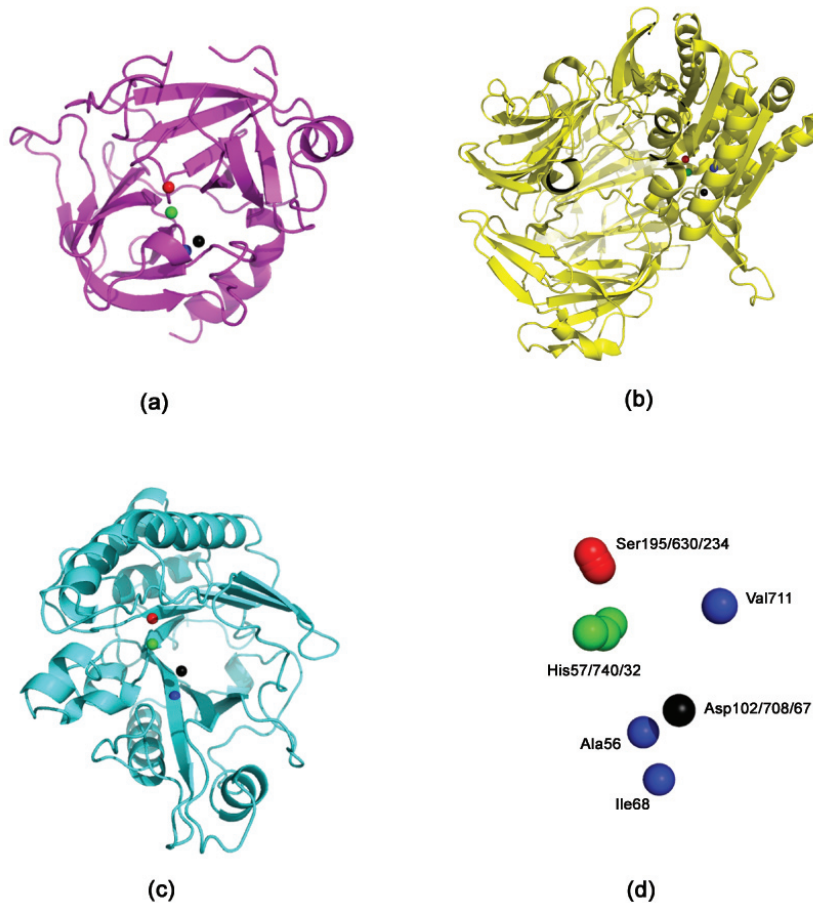


Figure 1. The active site residues in Trypsin, DPP4 and PI-PLC. (a) Trypsin (PDBid:1A0J) (b) DPP4 (PDBid:1N1M); (c) PI-PLC (PDBid:1PTD) (d) Superimposing the active site residues using DE-CAAF³⁵. The superimposition can be viewed in Superimposeproteins.p1m in [Dataset 1](#).

based on their catalytic residues³⁵. It can be seen that the closest non-polar residue to the catalytic triad in trypsin and PI-PLC (Ala56 in PDBid:1A0J, Ile68 in PDBid:1PTD) is differently placed from Val711 in DPP4 (PDBid:1N1M). This is also indicated by the greater RMSD (root mean square deviation) of the scaffold in PI-PLC to Motif2 as compared to Motif1. The differences in the position of peripheral residues is the source of the diverse specificities exhibited by these proteases. **Figure 2** shows the inhibition of PI-PLC using two gliptins - vildagliptin (LAF-237)²³ and K579²⁴. PI-PLC catalyzes hydrolysis of phospholipids to yield diacylglycerol and a phosphoryl alcohol. In the absence of inhibitors enzyme addition to the vesicle suspension causes an increase in turbidity due to vesicle aggregation (**Figure 2 a,c**). Aggregation in turn occurs as a result of formation of the enzyme endproduct diacylglycerol^{36,37}. A steady-state is reached under our conditions after 6–8 min. Addition of either LAF-237 (vildagliptin) or K579 leads to an obvious inhibition of the enzyme activity.

Dose-response curves for the inhibitors are shown in **Figure 2 (b,d)**. K579 is two orders of magnitude more potent than LAF-237 as a PI-PLC inhibitor, with half-maximal inhibitory concentrations IC_{50} respectively of 1 μ M and 100 μ M.

Phosphoinositide-specific phospholipase C inhibition data using the dipeptidyl peptidase-IV inhibitors K-579 and LAF-237

12 Data Files

<http://dx.doi.org/10.6084/m9.figshare.880620>

Querying a non-redundant set of human proteins using Motif1 and Motif2. Currently, the PDB database has about 25,000 human proteins. Using a identity cutoff of 50%, we chose a set of ~5000 proteins (**Supplementary Table 1**) as the target proteins.

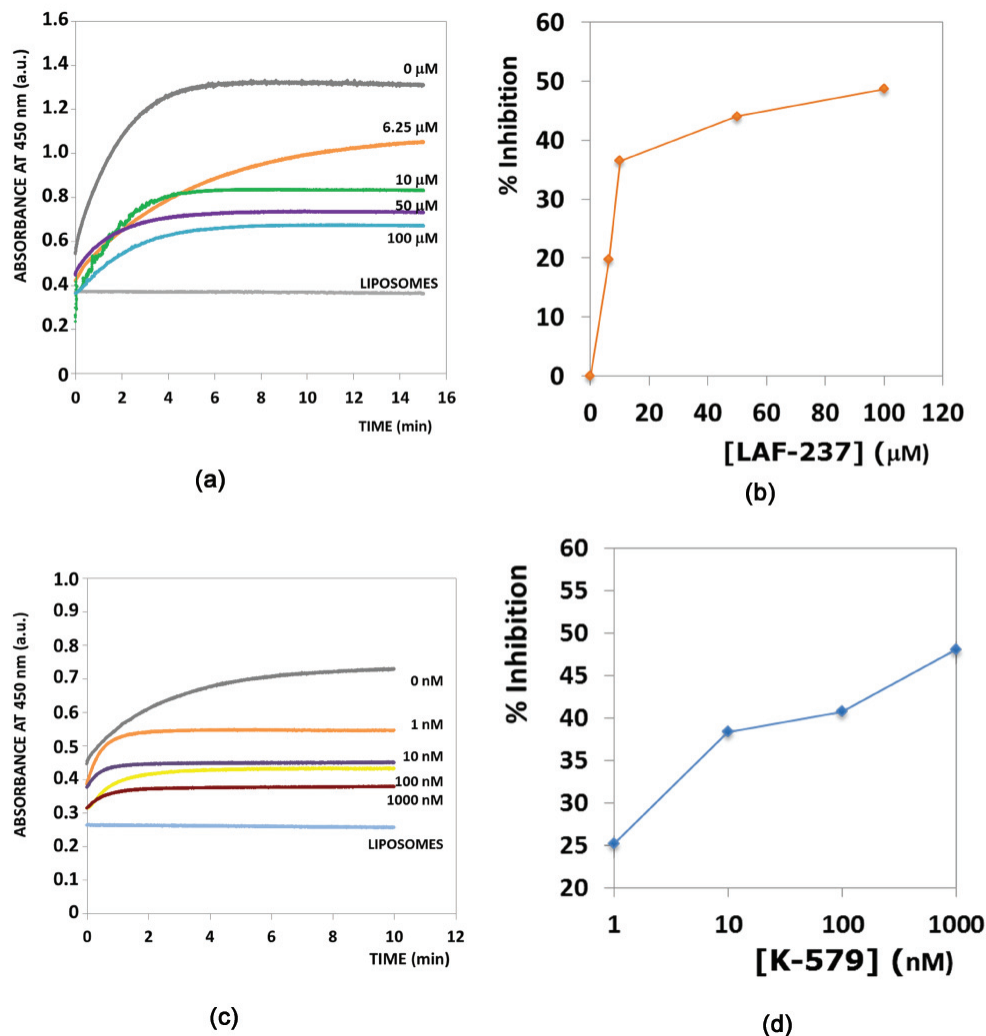


Figure 2. PI-PLC inhibition using DPP4 inhibitors. (a,c) Time courses of enzyme activity in the presence of varying amounts of inhibitors, respectively LAF-237 and K579. The trace marked LIPOSOMES corresponds to a control in the absence of PI-PLC. (b,d) Dose-response effect of inhibitors on PI-PLC activity. Activity was computed as the extent of vesicle aggregation after 10 min enzyme activity.

Table 2 shows ten proteins which have significant matches with Motif1 and Motif2. Given the context of lipases, acute pancreatitis and GLP-1 based therapies, we picked two proteins - the human pancreatic lipase-related protein 2 (PDBid:2OXE)²⁶ and a human gastric lipase (PDBid:1HLG)²⁷ - to demonstrate the distinct possibility that these proteins might be inhibited by DPP4 inhibitors. Table 1 shows the congruence of the DPP4 motif to these proteins using Motif1 and Motif2. It is interesting to note that the gastric lipase (PDBid:1HLG) has a good match with both motifs - Leu326 in PDBid:1HLG is congruent to Ala56 in PDBid:1A0J, and Ala237 (PDBid:1HLG) is congruent to Val711 (PDBid:1N1M).

Since both these proteins are lipases (hydrolases), this congruence to Motif1 and Motif2 is expected based on our previous results with PI-PLC²². However, our methodology also detects other proteins, often with a completely different enzymatic mechanism from hydrolases. A glutaminyl cyclase (PDBid:3PB4)³², a transferase, has a significantly congruent domain with Motif1 (lesser congruence with Motif2, as indicated by the RMSD) (Table 1). Figure 3 shows the proximity of the promiscuous scaffold to the active site of the cyclase, and also the congruence of the scaffold to Motif1.

Docking vildagliptin to the PIPLC structure. Since there are no DPP4 structures solved which ligand K-579, a DPP4 protein structure in complex with vildagliptin (PDBid:3W2TA)³⁸ was used to dock vildagliptin to the PIPLC structure complexed with myo-inositol (PDBid:1PTG³⁹) using DOCLASP⁴⁰ (Figure 4). The Pymol script for visualizing the docking (SupplementaryPymol.p1m) is provided as [Supplementary information](#).

Statistics of atoms making contact with inhibitors. There are 76 unique DPP4 inhibitors, defined by three letter codes, for which the

Table 2. Best matches in the set of ~5000 human proteins. (a) Motif1 (Asp102, Ser195, His57, Ala56) from Trypsin (b) Motif2 (Asp708, Ser630, His740, Val711) from DPP4.

| Motif | PDB | Description | CLASP Score |
|-------|------|---|-------------|
| 1 | 2ANY | Plasma kallikrein, light chain | 0.028 |
| 1 | 2OQ5 | Transmembrane protease, serine 11E | 0.037 |
| 1 | 3UOV | Lysophospholipase-like protein 1 | 0.041 |
| 1 | 2ODP | Complement C2 | 0.060 |
| 1 | 1IMJ | CCG1-interacting factor B | 0.065 |
| 1 | 3F6U | Vitamin K-dependent protein C heavy chain | 0.065 |
| 1 | 1ELV | Complement C1S component | 0.068 |
| 1 | 1MD8 | C1R complement serine protease | 0.068 |
| 1 | 1ORF | Granzyme A | 0.070 |
| 1 | 1FJ2 | Acyl protein thioesterase 1 | 0.071 |
| 2 | 1HLG | Gastric lipase | 0.042 |
| 2 | 1SPJ | Kallikrein 1 | 0.114 |
| 2 | 2F83 | Coagulation factor XI | 0.120 |
| 2 | 1ZJK | Mannan-binding lectin serine protease 2 | 0.131 |
| 2 | 3QLP | Thrombin light chain | 0.145 |
| 2 | 2QXI | Kallikrein-7 | 0.146 |
| 2 | 2XU7 | Histone-binding protein RBBP4 | 0.174 |
| 2 | 2W2N | Proprotein convertase subtilisin/kexin type 9 | 0.180 |
| 2 | 2HEH | KIF2C protein | 0.195 |
| 2 | 2ANY | Plasma kallikrein, light chain | 0.197 |

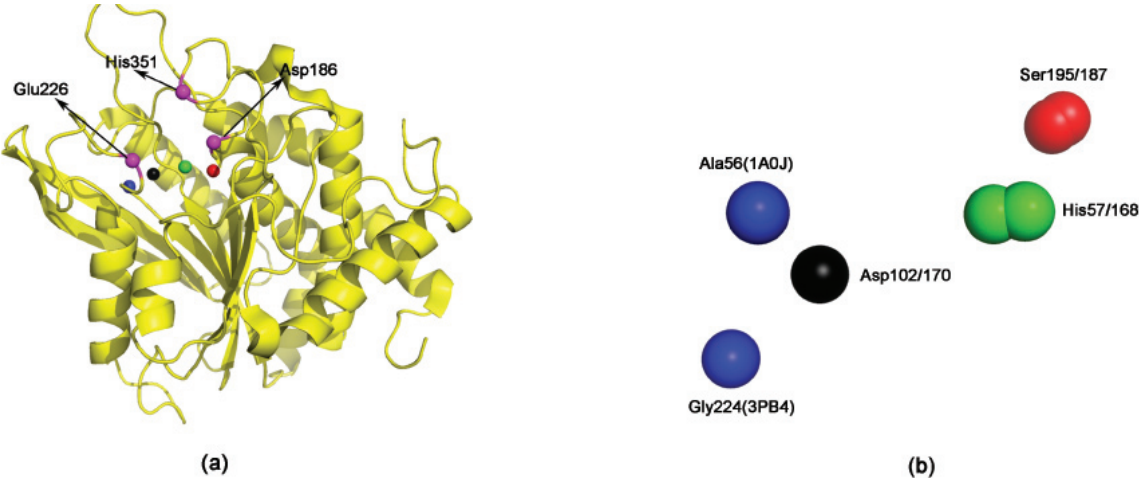


Figure 3. A scaffold congruent to the active site of Trypsin (PDBid:1A0J) in a glutaminyl cyclase (PDBid:3PB4). (a) The active site residues are marked in magenta. They are seen to be proximal to the identified scaffold. (b) Superimposition of Motif1 and the scaffold in glutaminyl cyclase. The exact pairwise interatomic distance and electrostatic potential differences are specified in Table 1.

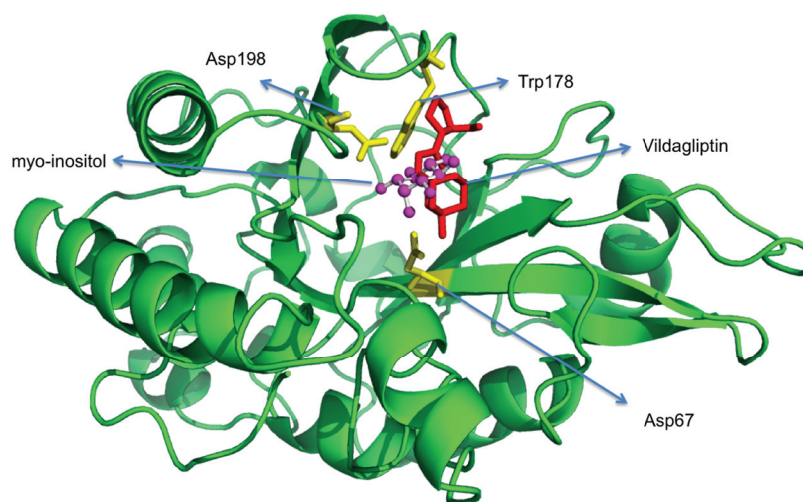


Figure 4. Docking vildagliptin to the PI-PLC structure in complex with myo-inositol (PDBid:1PTGA). Docking done using DOCLASP⁴⁰. The Pymol script for visualizing the docking (SupplementaryPymol.p1m) is provided as [Supplementary information](#).

ligand-DPP4 structure is solved ([Supplementary Table 2](#)). For uniformity, we chose the first four closest atoms from the protein that make contacts to the ligand, excluding hydrophobic interactions. [Table 3](#) shows the number of times each residue in DPP4 makes contact to the ligand. Three residues are ubiquitous in making contacts in all these ligands: Glu205, Glu206 and Tyr662 made contacts in 71, 68 and 63 ligands, respectively. Interestingly, Glu205 and Glu206 have been implicated as critical residues for the enzymatic activity of DPP4 through point mutations⁴¹. Note, that since only the first four residues were considered, these counts are conservative (and might be more). A recent study has found that inhibitors that bind to residues beyond the extensive subsite (defined as Val207, Ser209, Phe357 and Arg358) increases DPP4 inhibition, as compared to those inhibitors that form a covalent bond with Ser630³⁸. [Table 3](#) shows that very few inhibitors make such contacts. We created a library of motifs from these structures that can be used to query any protein using CLASP to determine the possibility that DPP4 inhibitors might bind to it ([Supplementary Table 3](#)), after removing equivalent ones to eliminate redundancy. This table shows the final list of 39 motifs (pruned from the initial 76); this is a comprehensive set of motifs that encapsulates the current knowledge about protein ligand interactions for the DPP4 enzyme. A facet of ligand binding that needs to be accounted for while choosing a motif is the spatial and electrostatic changes that can be induced by ligand binding. Thus, we obtain the residues involved in binding from the holo enzyme, but extract the motif values (pairwise distance and EPD) from the apo enzyme.

Discussion

The controversy regarding the side effects of the dpp4 inhibitors, particularly with respect to acute pancreatitis and pancreatic cancer, continues unabated. While some researchers feel that it is not acceptable to assume that 'absence of evidence is evidence of absence'^{42,43}, others believe that current data are not conclusive and the 'benefits by far outweigh the potential risks'¹⁶. Adding to the uncertainties are conflicting reports presented by different

groups²⁸⁻³¹. Notwithstanding the antagonistic views on the subject, it is unanimously accepted that current data are insufficient to establish a causal pathogenic effect of these drugs on such side effects⁴⁴.

Table 3. Number of times residues from the DPP4 enzyme ligand an inhibitor. Three residues - Glu205, Glu206 and Tyr662 - make contacts in 71, 68 and 63 ligands, respectively. Note, that since we only choose the first four residues based on proximity of the atoms closest to the ligand, these counts are conservative (and might be actually more).

| Residue | Number of ligands |
|---------|-------------------|
| ARG125 | 11 |
| GLU205 | 71 |
| GLU206 | 68 |
| VAL207 | 1 |
| SER209 | 3 |
| ARG358 | 6 |
| TYR547 | 18 |
| GLN553 | 1 |
| TYR585 | 1 |
| TRP629 | 1 |
| SER630 | 10 |
| TYR631 | 12 |
| TYR662 | 63 |
| ASN710 | 15 |

Various database studies have been undertaken in order to ascertain the effects of the GLP-1 therapies. Some studies ‘did not find an association between the use of exenatide or sitagliptin and acute pancreatitis’ with the caveat that the ‘limitations of this observational claims-based analysis cannot exclude the possibility of an increased risk’⁴⁵. On the other hand, other studies have shown that the use of ‘sitagliptin or exenatide increased the odds ratio for reported pancreatitis 6-fold as compared with other therapies’¹⁴. Further, they reported that ‘pancreatic cancer was more commonly reported among patients who took sitagliptin or exenatide as compared with other therapies’¹⁴. Although these studies concern the usage of both GLP-1 mimetics and the orally administered gliptins, and our study exclusively focusses on gliptins, and is not concerned with the GLP-1 mimetics data. The close relationship between chronic pancreatitis and pancreatic cancer is also a subject of intense research⁴⁶. Another administrative database study of US adults with type 2 diabetes reported increased odds of hospitalization for acute pancreatitis for patients undergoing GLP-1 based therapies sitagliptin¹³. Once again, such correlation of GLP-1 based therapies to acute pancreatitis is contested by other studies⁴⁷.

Our findings rationalize the elevated levels of serum lipase found in patients undergoing DPP4 inhibitor based therapies^{28,29}, keeping in mind that other studies contradict these reports^{30,31}. While several studies have reported that the GLP-1 mimetics do not induce pancreatitis in rats, mouse and/or monkey^{48–50}, these studies did not include DPP4 inhibitors, which are the compounds that might be responsible for interactions with pancreatic proteins according to our study. It is to be noted however that these mimetics may have other physiological effects and ‘the long-term consequences of sustained GLP-1 receptor activation in the human thyroid remain unknown and merit further investigation’⁵¹. Once again, the previous study⁵¹ has been challenged by another group who note that ‘findings previously reported in rodents may not apply to humans’⁵².

The orally administered gliptins differ in many aspects such as potency, excretion mechanism, target selectivity, half-life, metabolism and possible drug-drug interactions^{9,53,54}. This difference is also highlighted in the different concentrations of vildagliptin and K579 that inhibit PI-PLC. A recent study has also noted the differential off-target inhibition of enzymes by vildagliptin and sitagliptin using a high-throughput, multiplexed assay⁵⁵. Interestingly, the PI-PLC scaffold has a better match with the trypsin motif than with the DPP4 motif (Table 1). In order to be able to model these differences in our *in silico* search, it is important to be able to provide flexibility in the scoring mechanism.

To summarize, it has been noted in the case of GLP-1 based therapies that as ‘evidence of harm accumulates, but is vigorously discounted’ the ‘burden of proof now rests with those who wish to convince us of their safety’⁴³. Surveillance programs, real-life cohort studies and case-control studies can be supplemented by rational investigations of relevant proteins based on anecdotal reports⁵⁶. The methodology proposed in the current work, which specifically

demonstrates the effects of the DPP4 inhibitors, also presents a rational way of determining the inadvertent interactions of newly designed compounds with proteins, and thus prevent the recurrence of drug induced diseases being detected after considerable damage has already been inflicted on humans subjected to these drugs⁵⁷.

Materials and methods

In silico analysis

A comprehensive, non-redundant set of ~5000 human proteins (50% identity cutoff) was obtained from the PDB database⁵⁸. The CLASP package (<http://www.sanchak.com/clasp>) used for querying these proteins using motifs from trypsin and DPP4 is written in Perl on Ubuntu²⁰. Hardware requirements are modest - all results here are from a simple workstation (8GB ram), and runtimes for analyzing the ~5000 proteins was about 24 hours. Adaptive Poisson-Boltzmann Solver (APBS) and PDB2PQR packages were used to calculate the potential difference between the reactive atoms of the corresponding proteins^{59,60}. The APBS parameters and electrostatic potential units were set as described previously in Chakraborty *et al.*²⁰. All protein structures were rendered by PyMol (<http://www.pymol.org/>). Protein structures have been superimposed using MUSTANG³⁴ and DECAAF³⁵.

Protein, substrate and reagents

PI-PLC was purchased from Sigma. Vildagliptin (LAF-237) was obtained from Selleckchem, and K579 was obtained from Santa Cruz.

PI-PLC assay and inhibition using DPP4 inhibitors

Vesicle preparation and characterization. The appropriate lipids were mixed in organic solution, and the solvent was evaporated to dryness under N₂. Solvent traces were removed by evacuating the lipids for at least 2 hours. The lipids were then swollen in 10 mM Hepes, 150 mM NaCl, pH 7.5 buffer. Large unilamellar vesicles (LUV) were prepared from the swollen lipids by extrusion and sized by using 0.1 μ m poresize Nuclepore filters, as described by Ahlyayach *et al.*³⁶. LUV composition was egg phosphatidylcholine: egg phosphatidylethanolamine: cholesterol at a 2:1:1 mole ratio. The average size of LUV was measured by quasi-elastic light scattering, using a Malvern Zeta-sizer instrument. Lipid concentration, determined by phosphate analysis, was 0.3 mM in all experiments.

Aggregation Assay. Enzyme activity was assayed measuring enzyme-induced vesicle aggregation. All assays were carried out at 39°C with continuous stirring, in 10 mM Hepes, 150 mM NaCl buffer (pH 7.5), in the presence of 0.1% BSA for optimum catalytic activity. Enzyme concentration was 0.16 U/mL, and liposomal concentration was 0.3 mM. Lipid aggregation was monitored in a Cary Varian UV-vesicle spectrometer as an increase in turbidity (absorbance at 450 nm) of the sample, as described by Villar *et al.*³⁷. The data are average values of two closely similar experiments.

Analyzing known DPP4 inhibitors with solved structures. In order to obtain all known structures of DPP4 with inhibitors bound to the

active site, we did a search for the keyword dipeptidyl-peptidase on the PDB database, and choose proteins with DPP4 inhibitors as ligands. There are 76 such unique compounds (defined by three letter codes) that are reported to date (May 2014). We docked the DPP4 inhibitor to the PIPLC active site using DOCLASP⁴⁰.

Data availability

figshare: Phosphoinositide-specific phospholipase C inhibition data using the dipeptidyl peptidase-IV inhibitors K-579 and LAF-237, <http://dx.doi.org/10.6084/m9.figshare.880620>

Author contributions

SC, ARR and BA performed the experiments. All authors analyzed the data, and contributed equally to the writing and subsequent refinement of the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

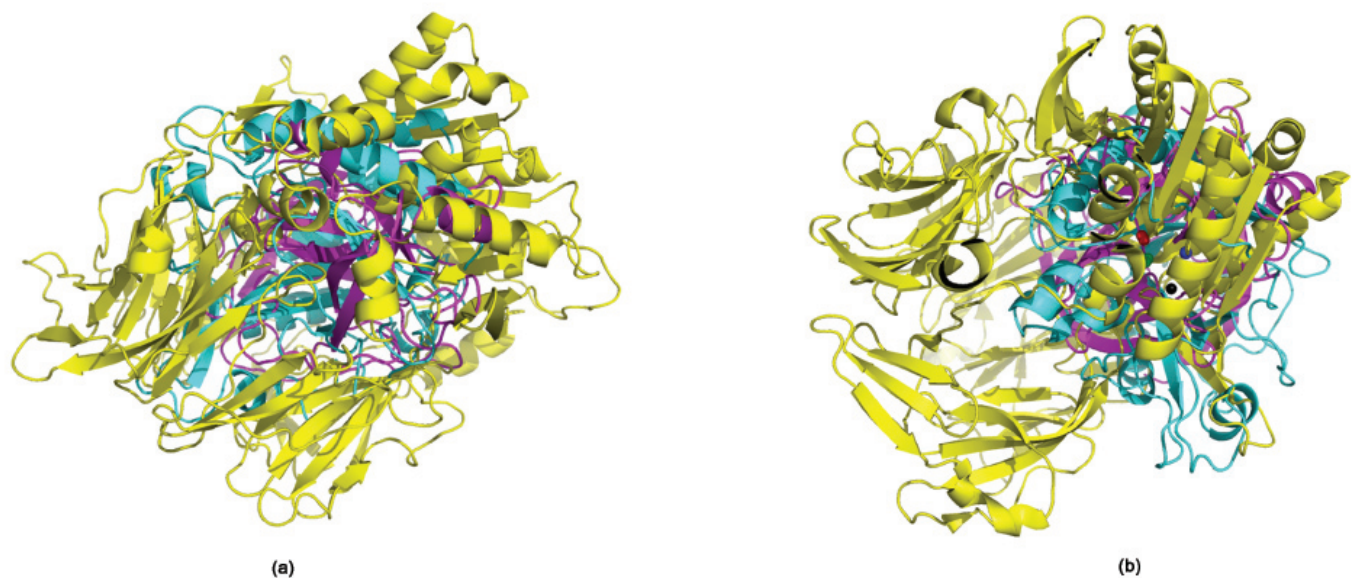
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Supplementary information

Supplementary Pymol scripts. Click here to access the files. <http://dx.doi.org/10.5256/f1000research.3002.s40929>



Supplementary Figure 1. Superimposition of trypsin (PDBid:1A0J - magenta), dipeptidyl peptidase-IV (PDBid:1N1M - yellow) and phosphoinositide-specific phospholipase C (PDBid:1PTD - cyan). It is seen that there is no structural similarity in the two proteins. (a) Using MUSTANG³⁴. (b) Using DECAAF³⁵.

Supplementary Table 1. PDB IDs of ~5000 human proteins analyzed in this study.

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 3KVO 3KVQ 3KVW 3KW6 3KY9 3KZ8 3KZD 3L00 3L11 3L15 3L1X 3L2C 3L2P 3L3C 3L42 3L43 3L46
 3L4C 3L4G 3L4H 3L4Y 3L50 3L5H 3L5I 3L5K 3L6A 3L6B 3L6X 3L81 3L9Q 3LCY 3LD6 3LE4 3LF5 3LFV
 3LGD 3LH5 3LHR 3LJB 3LJU 3LJW 3LK9 3LL6 3LLH 3LLK 3LLM 3LLP 3LLU 3LM5 3LMN 3LNY
 3LOF 3LPW 3LQ9 3LQH 3LQM 3LQV 3LRA 3LRE 3LRI 3LRN 3LRQ 3LRR 3LRU 3LS8 3LUC 3LUI
 3LVR 3LWE 3LWK 3LXX 3LY5 3LYR 3LZB 3M03 3M06 3M1D 3M5B 3M66 3M7P 3M9J 3MAO 3MAX
 3MAZ 3MB3 3MB4 3MBY 3MCB 3MCE 3MCF 3MDA 3MDC 3MDF 3MDG 3MDI 3MDM 3MEW 3MFK
 3MGH 3MGI 3MIJ 3MJG 3MJK 3MK1 3MK4 3MK6 3MMY 3MNG 3MOP 3MOS 3MPX 3MQ4 3MQ7
 3MQI 3MQL 3MQM 3MQP 3MR2 3MR3 3MR5 3MR6 3MSH 3MT5 3MTC 3MTR 3MTS 3MTT 3MU6
 3MUJ 3MUM 3MUP 3MUR 3MUT 3MUV 3MVA 3MVB 3MWD 3MX7 3MXH 3MXN 3MXO 3MYI 3N00

Supplementary Table 2. Residues of DPP4 closest to the bound ligand with possible hydrogen bonds.

Interactions sorted based on the distance. N: Number of atoms in the ligand, R/A/LA/D: Residue number/Atom of the residue/Atom of ligand/distance between the interacting atoms (in Å). For example, 'E205/OE1/N25/2.7' means that the atom OE1 from Glu205 is at 2.7 Å from the N25 atom of W94 in PDBid:3VJLA. For uniformity, we choose the first four closest atoms. This might result in choosing some atoms which are unlikely to form a hydrogen bond (for example, in PDBid:4J3JA S209/OG is at 4.8 Å from NAQ).

| PDB | HET | N | R/A/LA/D | R/A/LA/D | R/A/LA/D | R/A/LA/D |
|-------|-----|----|------------------|------------------|------------------|------------------|
| 3VJLA | W94 | 33 | E205/OE1/N25/2.7 | E206/OE1/N25/2.8 | N710/ND2/O33/2.9 | Y662/OH/O33/3 |
| 2AJ8A | SC3 | 26 | E205/OE2/N13/2.7 | E206/OE1/N13/3 | Y631/N/O23/3.1 | Y547/OH/N7/3.4 |
| 2RGUA | 356 | 35 | E205/OE2/N27/3 | Y662/OH/N27/3.1 | Y631/N/O10/3.1 | E206/OE2/N27/3.1 |
| 4A5SA | N7F | 37 | E205/OE2/N18/2.7 | E206/OE2/N18/2.7 | Y662/OH/N18/2.8 | Y631/N/O26/3 |
| 2QTBA | 474 | 32 | E205/OE2/N6/2.7 | N710/ND2/O7/2.8 | E206/OE1/N6/2.8 | R358/NE/N56/2.8 |
| 2OGZA | U1N | 24 | Y631/N/O25/3 | E206/OE1/N12/3.1 | R125/NH2/O15/3.1 | E205/OE2/O15/3.3 |
| 2JIDA | GVB | 24 | E206/OE2/N20/2.8 | E205/OE2/N20/2.9 | Y662/OH/N20/3 | R125/NH1/O25/3.8 |
| 2I78B | KIQ | 31 | Y662/OH/N/3 | E205/O/O/4.1 | E206/OE1/O/4.2 | R669/NH2/O/4.4 |
| 3H0CA | PS4 | 32 | E205/OE2/N21/2.7 | Y662/OH/N21/2.9 | E206/OE2/N21/2.9 | Q553/N/O3/3 |
| 2AJLI | JNH | 24 | S630/OG/N3/2.3 | E206/OE2/N2/2.5 | Y547/OH/N3/2.6 | E205/OE2/N2/2.7 |
| 2BUBA | FPB | 28 | E206/OE2/N18/2.5 | E205/OE2/N18/2.9 | Y662/OH/N18/3 | Y547/OH/O16/4.3 |
| 4DSAA | D1C | 29 | E206/OE2/NAY/2.6 | E205/O/OBC/3.1 | Y662/OH/NAY/3.1 | Y585/OH/NAI/3.9 |
| 2OPHA | 277 | 23 | E205/OE2/N33/2.7 | N710/ND2/O32/2.8 | Y662/OH/N33/3.1 | E206/OE2/N33/3.1 |
| 1RWQA | 5AP | 27 | Y662/OH/N21/2.5 | E206/OE2/N21/2.8 | E205/OE2/N23/2.9 | R125/NH2/N1/3.4 |
| 2QJRA | PZF | 29 | E205/OE2/N20/2.6 | R358/NE/O18/2.8 | E206/OE2/N20/2.9 | Y662/OH/N20/3 |
| 2FJPA | S14 | 31 | E205/OE2/N30/2.7 | N710/ND2/O32/2.8 | E206/OE2/N30/2.8 | Y547/OH/O33/2.8 |
| 2OAEA | AIL | 21 | E203/OE2/N2/2.8 | E204/OE2/N2/2.8 | N711/ND2/O8/3.1 | Y663/OH/N9/3.1 |
| 3G0CA | RUF | 27 | E205/OE1/N9/3 | E206/OE1/N9/3.2 | Y631/N/O23/3.4 | Y547/OH/N12/3.6 |
| 3C43A | 315 | 31 | E205/OE2/N6/2.8 | Y662/OH/N6/3 | N710/ND2/O5/3 | E206/OE2/N6/3 |
| 3BJMA | BJM | 23 | S630/OG/N23/2.4 | E205/OE2/N7/2.7 | E206/OE2/N7/2.7 | Y547/OH/O15/2.8 |
| 3O95A | 01T | 26 | E206/OE2/N13/2.5 | E205/OE1/N13/2.8 | Y662/OH/N13/2.8 | R125/NH1/O19/3 |
| 3G0GA | RUM | 24 | E205/OE1/N24/2.9 | E206/OE1/N24/3.1 | Y631/N/O8/3.2 | R125/NH2/N17/3.3 |
| 2G5PA | ADF | 29 | S630/OG/N22/2.4 | Y662/OH/N8/3.1 | Y547/OH/N22/3.1 | E206/OE2/N7/3.1 |
| 2BUC | 008 | 26 | E206/OE2/N10/2.7 | Y662/OH/N10/2.8 | E205/OE2/N10/3 | Y547/OH/O13/4.5 |
| 2QOEA | 448 | 29 | E206/OE2/N20/2.7 | E205/OE2/N20/2.9 | Y662/OH/N20/2.9 | Y547/OH/O22/4.6 |
| 2OLEA | KR2 | 30 | E206/OE2/NAM/2.7 | Y662/OH/NAM/3.6 | E205/OE2/NAM/4 | Y547/OH/OAP/4.5 |
| 3KWFA | B1Q | 27 | E205/OE2/N21/2.7 | N710/ND2/O19/2.7 | Y662/OH/N21/3 | R125/NH2/O19/3 |
| 3SX4A | KXA | 58 | Y662/OH/N25/2.7 | E206/OE2/N25/2.7 | E205/OE2/N25/2.8 | R125/NH1/O26/3.1 |
| 2ONCA | SY1 | 27 | E205/OE1/N1/2.6 | Y631/N/O17/3.1 | Y547/OH/N18/3.2 | E206/OE1/N1/3.4 |
| 2I03B | AXD | 29 | S630/OG/N14/2.4 | E206/OE1/N1/2.8 | Y662/OH/O16/2.9 | Y547/OH/N14/3 |
| 3KWJA | 23Q | 27 | E205/OE2/N17/2.6 | Y662/OH/N17/2.8 | E206/OE2/N17/2.8 | S209/OG/O19/3.3 |
| 3CCCA | 7AC | 21 | E205/OE1/N20/2.5 | Y662/OH/N20/2.7 | E206/OE2/N20/3.2 | Y631/N/N9/3.3 |
| 3SWWA | KXB | 25 | E205/OE2/N21/2.7 | Y662/OH/N21/2.8 | E206/OE2/N21/2.9 | R125/NH2/N19/3.5 |
| 4G1FA | OWG | 24 | E206/OE2/N9/2.8 | Y662/OH/N9/2.9 | Y547/OH/N2/3.1 | Y631/N/O20/3.1 |
| 3C45A | 317 | 30 | E205/OE2/N6/2.8 | E206/OE2/N6/2.8 | Y662/OH/N6/3 | Y547/OH/N29/3.7 |

| PDB | HET | N | R/A/LA/D | R/A/LA/D | R/A/LA/D | R/A/LA/D |
|-------|-----|----|------------------|------------------|-------------------|------------------|
| 2G63B | AAF | 29 | S630/OG/N18/2.4 | E205/OE2/N7/2.6 | Y662/OH/N8/3.1 | Y547/OH/N18/3.1 |
| 1X70A | 715 | 28 | E206/OE2/N20/2.7 | E205/OE2/N20/2.8 | Y662/OH/N20/2.8 | S209/OG/N27/3.9 |
| 2GBIA | XIH | 29 | E204/OE2/N14/2.3 | Y632/N/O/2.8 | E203/OE2/N14/3 | Y663/OH/N14/3.1 |
| 3G0BA | T22 | 25 | E205/OE1/N13/2.5 | R125/NH2/N24/3.1 | Y631/N/O26/3.2 | E206/OE1/N13/3.3 |
| 2IITA | 872 | 28 | E205/OE2/N20/2.7 | Y662/OH/N20/2.8 | E206/OE2/N20/2.9 | N710/OD1/N20/4.5 |
| 4JH0A | 1MD | 27 | Y662/OH/N16/2.7 | E206/OE2/N16/2.7 | Y547/OH/O1/2.8 | E205/OE2/N16/2.9 |
| 4LKOA | 1WH | 25 | Y662/OH/N/2.7 | E206/OE2/N/2.8 | E205/OE2/N/2.9 | Y547/OH/O2/3 |
| 2RIPA | 34Q | 25 | N710/ND2/O1/2.6 | E205/OE2/N3/2.8 | Y662/OH/N3/2.8 | E206/OE1/N3/2.8 |
| 3QBJA | NXZ | 25 | Y662/OH/N18/2.7 | E206/OE2/N18/2.7 | N710/ND2/O25/2.8 | E205/OE2/N18/2.9 |
| 3HACA | 361 | 23 | Y662/OH/N23/2.7 | E205/OE2/N23/2.8 | E206/OE1/N12/4.2 | N710/OD1/N23/4.3 |
| 3VJMA | W61 | 32 | E205/OE1/N28/2.7 | E206/OE1/N28/2.7 | Y662/OH/O57/2.9 | N710/ND2/O57/2.9 |
| 3O9VA | 10T | 23 | Y547/OH/O15/2.5 | E206/OE2/N19/2.6 | Y662/OH/N19/2.7 | E205/OE1/N19/2.8 |
| 4DSZA | DC3 | 26 | E206/OE2/NAM/2.8 | E205/OE2/NAM/3.1 | Y662/OH/NAM/3.1 | S209/OG/NAR/4.7 |
| 4J3JA | D3C | 30 | E206/OE2/NAM/2.8 | E205/OE2/NAM/3.1 | Y662/OH/NAM/3.3 | S209/OG/NAQ/4.8 |
| 4DTCA | D5C | 33 | E206/OE2/NAM/2.7 | E205/OE2/NAM/3.1 | Y662/OH/NAM/3.3 | R669/NH2/OAQ/4.2 |
| 3OPMA | LUI | 28 | E205/OE1/N18/2.7 | Y662/OH/N18/2.8 | E206/OE2/N18/2.9 | W629/O/N27/3 |
| 2OAGB | DLI | 31 | E205/OE2/N22/2.5 | Y662/OH/N22/2.6 | E206/OE2/N22/2.9 | R358/NE/O1/3.2 |
| 2GBGA | 1AD | 19 | S631/OG/N12/2.4 | E203/OE2/N14/2.7 | Y548/OH/N12/3 | E204/OE2/N14/3.1 |
| 2HHAA | 3TP | 26 | E205/OE2/N6/2.7 | E206/OE2/N6/2.7 | Y662/OH/N6/2.9 | N710/ND2/O5/2.9 |
| 2QT9A | 524 | 31 | E205/OE2/N19/2.6 | E206/OE2/N19/2.8 | Y662/OH/N19/2.9 | N710/ND2/O20/2.9 |
| 2OQVA | MA9 | 32 | E206/OE2/N27/2.7 | Y662/OH/N27/3 | R358/NE/O4/3.1 | E205/OE2/N27/3.3 |
| 3F8SA | PF2 | 26 | E205/OE2/N3/2.5 | Y662/OH/O7/2.8 | N710/OD1/O7/3 | E206/OE2/N3/3.2 |
| 2AJBA | 0QG | 24 | S630/OG/N2/2.4 | E205/OE2/N/2.7 | HIS740/NE2/O2/2.9 | Y662/OH/O/3 |
| 2G5TA | ACF | 26 | S630/OG/N22/2.4 | E205/OE2/N7/2.6 | Y662/OH/O3/3 | N710/ND2/O3/3 |
| 3NOXA | 6A5 | 28 | E205/OE2/N16/2.4 | E206/OE2/N16/2.7 | Y662/OH/N16/3 | R125/NH2/N4/3.7 |
| 3W2TA | LF7 | 22 | S630/OG/N2/2.4 | E205/OE1/N12/2.8 | Y662/OH/O20/3 | E206/OE2/N12/3 |
| 3D4LA | 605 | 26 | E205/OE2/N15/2.6 | R358/NE/O42/2.8 | Y662/OH/N15/2.9 | V207/O/N41/2.9 |
| 2QKYA | 13Z | 26 | S630/OG/O2/2.1 | E205/O/O4/2.5 | Y547/OH/O2/2.6 | E206/OE1/O4/2.8 |
| 3Q8WA | AZV | 38 | R125/NH1/O/2.5 | E206/OE2/NAG/2.6 | Y662/OH/NAG/2.8 | E205/OE2/NAG/2.9 |
| 3EIOA | AJH | 33 | Y585/OH/OBD/2.6 | E205/OE2/NBG/2.7 | Y662/OH/NBG/2.9 | E206/OE2/NBG/2.9 |
| 3Q0TA | LGE | 26 | Y662/OH/N21/2.6 | E205/OE2/N21/2.7 | E206/OE2/N21/2.9 | R125/NH1/O22/3.4 |
| 2P8SA | 417 | 58 | E205/OE2/N38/2.8 | E206/OE2/N38/2.9 | Y662/OH/N38/3.2 | S209/OG/N34/3.4 |
| 2OQIB | GGO | 28 | E205/OE2/N/2.4 | Y662/OH/N/2.7 | E206/OE2/N/2.9 | R358/NE/O/3.2 |
| 4PNZA | 2VH | 28 | E205/OE2/N/2.7 | E206/OE2/N/2.8 | Y662/OH/N/2.9 | Y547/OH/O/3.2 |
| 3VJKA | M51 | 30 | E205/OE1/N21/2.9 | Y662/OH/O30/2.9 | E206/OE1/N21/2.9 | N710/ND2/O30/3 |
| 3OC0A | B2Q | 23 | E205/OE2/NS/2.8 | S209/OG/OB/2.9 | Y662/OH/NS/3.4 | E206/OE2/NS/3.5 |
| 4N8DA | 2KS | 24 | E206/OE2/N10/2.7 | E205/OE2/N10/2.8 | Y662/OH/N10/2.8 | N710/OD1/N10/4.5 |
| 4N8EA | 2KV | 22 | E205/OE2/N15/2.7 | E206/OE2/N15/2.7 | Y662/OH/N15/2.8 | N710/OD1/N15/4.3 |
| 2IIVA | 565 | 24 | E205/OE2/N20/2.7 | Y662/OH/N20/2.8 | E206/OE2/N20/2.9 | N710/ND2/N20/4.4 |
| 3HABA | 677 | 27 | E205/OE2/N23/2.7 | Y662/OH/N23/2.7 | E206/OE1/N12/4.3 | N710/OD1/N23/4.4 |
| 2I3ZA | LIR | 27 | E203/OE2/N18/2.5 | Y632/N/O9/2.7 | Y548/OH/N6/3.4 | E204/OE2/N18/3.4 |

Supplementary Table 3. Library of non-redundant motifs. This library of motifs can be used to query any protein using CLASP to determine the possibility that DPP4 inhibitors might bind to it.

| PDB | Motif Name | Motif |
|-------|------------|--|
| 3VJLA | 2OQVA1 | GLU205/OE1 GLU206/OE1 TYR662/OH ASN710/ND2 |
| 2AJ8A | 2OQVA2 | GLU205/OE2 GLU206/OE1 TYR547/OH TYR631/N |
| 2RGUA | 2OQVA3 | GLU205/OE2 GLU206/OE2 TYR631/N TYR662/OH |
| 2QTBA | 2OQVA4 | GLU205/OE2 GLU206/OE1 ARG358/NE ASN710/ND2 |
| 2OGZA | 2OQVA5 | ARG125/NH2 GLU205/OE2 GLU206/OE1 TYR631/N |
| 2JIDA | 2OQVA6 | ARG125/NH1 GLU205/OE2 GLU206/OE2 TYR662/OH |
| 2I78B | 2OQVA7 | GLU205/O GLU206/OE1 TYR662/OH ARG669/NH2 |
| 3H0CA | 2OQVA8 | GLU205/OE2 GLU206/OE2 GLN553/N TYR662/OH |
| 2AJLI | 2OQVA9 | GLU205/OE2 GLU206/OE2 TYR547/OH SER630/OG |
| 2BUBA | 2OQVA10 | GLU205/OE2 GLU206/OE2 TYR547/OH TYR662/OH |
| 4DSAA | 2OQVA11 | GLU205/O GLU206/OE2 TYR585/OH TYR662/OH |
| 2OPHA | 2OQVA12 | GLU205/OE2 GLU206/OE2 TYR662/OH ASN710/ND2 |
| 1RWQA | 2OQVA13 | ARG125/NH2 GLU205/OE2 GLU206/OE2 TYR662/OH |
| 2QJRA | 2OQVA14 | GLU205/OE2 GLU206/OE2 ARG358/NE TYR662/OH |
| 2FJPA | 2OQVA15 | GLU205/OE2 GLU206/OE2 TYR547/OH ASN710/ND2 |
| 2OAEA | 2OQVA16 | GLU205/OE2 GLU206/OE2 TYR662/OH ASN711/ND2 |
| 3G0CA | 2OQVA17 | GLU205/OE1 GLU206/OE1 TYR547/OH TYR631/N |
| 3O95A | 2OQVA18 | ARG125/NH1 GLU205/OE1 GLU206/OE2 TYR662/OH |
| 3G0GA | 2OQVA19 | ARG125/NH2 GLU205/OE1 GLU206/OE1 TYR631/N |
| 2G5PA | 2OQVA20 | GLU206/OE2 TYR547/OH SER630/OG TYR662/OH |
| 3KWFA | 2OQVA21 | ARG125/NH2 GLU205/OE2 TYR662/OH ASN710/ND2 |
| 2I03B | 2OQVA22 | GLU206/OE1 TYR547/OH SER630/OG TYR662/OH |
| 3KWJA | 2OQVA23 | GLU205/OE2 GLU206/OE2 SER209/OG TYR662/OH |
| 3CCCA | 2OQVA24 | GLU205/OE1 GLU206/OE2 TYR631/N TYR662/OH |
| 4G1FA | 2OQVA25 | GLU206/OE2 TYR547/OH TYR631/N TYR662/OH |
| 2G63B | 2OQVA26 | GLU205/OE2 TYR547/OH SER630/OG TYR662/OH |
| 2IITA | 2OQVA27 | GLU205/OE2 GLU206/OE2 TYR662/OH ASN710/OD1 |
| 2RIPA | 2OQVA28 | GLU205/OE2 GLU206/OE1 TYR662/OH ASN710/ND2 |
| 3HACA | 2OQVA29 | GLU205/OE2 GLU206/OE1 TYR662/OH ASN710/OD1 |
| 3O9VA | 2OQVA30 | GLU205/OE1 GLU206/OE2 TYR547/OH TYR662/OH |
| 4DTCA | 2OQVA31 | GLU205/OE2 GLU206/OE2 TYR662/OH ARG669/NH2 |
| 3OPMA | 2OQVA32 | GLU205/OE1 GLU206/OE2 TRP629/O TYR662/OH |
| 2AJBA | 2OQVA33 | GLU205/OE2 SER630/OG TYR662/OH HIS740/NE2 |
| 2G5TA | 2OQVA34 | GLU205/OE2 SER630/OG TYR662/OH ASN710/ND2 |
| 3W2TA | 2OQVA35 | GLU205/OE1 GLU206/OE2 SER630/OG TYR662/OH |
| 3D4LA | 2OQVA36 | GLU205/OE2 VAL207/O ARG358/NE TYR662/OH |
| 2QKYA | 2OQVA37 | GLU205/O GLU206/OE1 TYR547/OH SER630/OG |
| 3EIOA | 2OQVA38 | GLU205/OE2 GLU206/OE2 TYR585/OH TYR662/OH |
| 2I3ZA | 2OQVA39 | GLU205/OE2 GLU206/OE2 TYR547/OH TYR631/N |

References

1. Elrick H, Stummler L, Hlad CJ, *et al.*: **Plasma insulin response to oral and intravenous glucose administration.** *J Clin Endocrinol Metab.* 1964; **24**(10): 1076–1082.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. Baggio LL, Drucker DJ: **Biology of incretins: GLP-1 and GIP.** *Gastroenterology.* 2007; **132**(6): 2131–2157.
[PubMed Abstract](#) | [Publisher Full Text](#)
3. Mentlein R, Gallwitz B, Schmidt WE: **Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum.** *Eur J Biochem.* 1993; **214**(3): 829–835.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Nauck M, Stockmann F, Ebert R, *et al.*: **Reduced incretin effect in type 2 (non-insulin-dependent) diabetes.** *Diabetologia.* 1986; **29**(1): 46–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
5. Drucker DJ, Nauck MA: **The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes.** *Lancet.* 2006; **368**(9548): 1696–1705.
[PubMed Abstract](#) | [Publisher Full Text](#)
6. Green BD, Flatt PR: **Incretin hormone mimetics and analogues in diabetes therapeutics.** *Best Pract Res Clin Endocrinol Metab.* 2007; **21**(4): 497–516.
[PubMed Abstract](#) | [Publisher Full Text](#)
7. Holst JJ, Deacon CF: **Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes.** *Diabetes.* 1998; **47**(11): 1663–1670.
[PubMed Abstract](#) | [Publisher Full Text](#)
8. Dicker D: **DPP-4 inhibitors: impact on glycemic control and cardiovascular risk factors.** *Diabetes Care.* 2011; **34**(Suppl 2): S276–278.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Ahren B: **DPP-4 inhibitors.** *Best Pract Res Clin Endocrinol Metab.* 2007; **21**(4): 517–533.
[PubMed Abstract](#) | [Publisher Full Text](#)
10. Mentlein R: **Dipeptidyl-peptidase IV (CD26)–role in the inactivation of regulatory peptides.** *Regul Pept.* 1999; **85**(1): 9–24.
[PubMed Abstract](#) | [Publisher Full Text](#)
11. Wesley UV, McGroarty M, Homoyouni A: **Dipeptidyl peptidase inhibits malignant phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway.** *Cancer Res.* 2005; **65**(4): 1325–1334.
[PubMed Abstract](#) | [Publisher Full Text](#)
12. Havre PA, Abe M, Urasaki Y, *et al.*: **The role of CD26/dipeptidyl peptidase IV in cancer.** *Front Biosci.* 2008; **13**: 1634–1645.
[PubMed Abstract](#) | [Publisher Full Text](#)
13. Singh S, Chang HY, Richards TM, *et al.*: **Glucagonlike peptide 1-based therapies and risk of hospitalization for acute pancreatitis in type 2 diabetes mellitus: a population-based matched case-control study.** *JAMA Intern Med.* 2013; **173**(7): 534–539.
[PubMed Abstract](#) | [Publisher Full Text](#)
14. Elashoff M, Matveyenko AV, Gier B, *et al.*: **Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies.** *Gastroenterology.* 2011; **141**(1): 150–156.
[PubMed Abstract](#) | [Publisher Full Text](#)
15. Matveyenko AV, Dry S, Cox HI, *et al.*: **Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin.** *Diabetes.* 2009; **58**(7): 1604–1615.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
16. Nauck MA: **A critical analysis of the clinical use of incretin-based therapies: The benefits by far outweigh the potential risks.** *Diabetes Care.* 2013; **36**(7): 2126–2132.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Drucker DJ, Sherman SI, Bergenstal RM, *et al.*: **The safety of incretin-based therapies–review of the scientific evidence.** *J Clin Endocrinol Metab.* 2011; **96**(7): 2027–2031.
[PubMed Abstract](#) | [Publisher Full Text](#)
18. Scheen AJ: **Cardiovascular effects of dipeptidyl peptidase-4 inhibitors: from risk factors to clinical outcomes.** *Postgrad Med.* 2013; **125**(3): 7–20.
[PubMed Abstract](#) | [Publisher Full Text](#)
19. Hu Y, Bajorath J: **High-resolution view of compound promiscuity [v2; ref status: indexed, <http://f1000r.es/t1g>].** *F1000Res.* 2013; **2**: 144.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
20. Chakraborty S, Minda R, Salaye L, *et al.*: **Active site detection by spatial conformity and electrostatic analysis-unravelling a proteolytic function in shrimp alkaline phosphatase.** *PLoS One.* 2011; **6**(12): e28470.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Chakraborty S, Asgeirsson B, Minda R, *et al.*: **Inhibition of a cold-active alkaline phosphatase by imipenem revealed by *in silico* modeling of metallo- β -lactamase active sites.** *FEBS Lett.* 2012; **586**(20): 3710–3715.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Rendon-Ramirez A, Shukla M, Oda M, *et al.*: **A computational module assembled from different protease family motifs identifies PI PLC from *Bacillus cereus* as a putative prolyl peptidase with a serine protease scaffold.** *PLoS One.* 2013; **8**(8): e70923.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Villhauer EB, Brinkman JA, Naderi GB, *et al.*: **1-[[[3-hydroxy-1-adamantyl]amino]acetyl]-2-cyano-(S)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties.** *J Med Chem.* 2003; **46**(13): 2774–2789.
[PubMed Abstract](#) | [Publisher Full Text](#)
24. Takasaki K, Iwase M, Nakajima T, *et al.*: **K579, a slow-binding inhibitor of dipeptidyl peptidase IV is a long-acting hypoglycemic agent.** *Eur J Pharmacol.* 2004; **486**(3): 335–342.
[PubMed Abstract](#) | [Publisher Full Text](#)
25. Rasmussen HB, Branner S, Wiberg FC, *et al.*: **Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog.** *Nat Struct Biol.* 2003; **10**(1): 19–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
26. Eydoux C, Spinelli S, Davis TL, *et al.*: **Structure of human pancreatic lipase-related protein 2 with the lid in an open conformation.** *Biochemistry.* 2008; **47**(36): 9553–9564.
[PubMed Abstract](#) | [Publisher Full Text](#)
27. Roussel A, Canaan S, Egloff MP, *et al.*: **Crystal structure of human gastric lipase and model of lysosomal acid lipase, two lipolytic enzymes of medical interest.** *J Biol Chem.* 1999; **274**(24): 16995–17002.
[PubMed Abstract](#) | [Publisher Full Text](#)
28. Tokuyama H, Kawamura H, Fujimoto M, *et al.*: **A low-grade increase of serum pancreatic exocrine enzyme levels by dipeptidyl peptidase-4 inhibitor in patients with type 2 diabetes.** *Diabetes Res Clin Pract.* 2013; **100**(3): e66–e69.
[PubMed Abstract](#) | [Publisher Full Text](#)
29. Lando HM, Alattar M, Dua AP: **Elevated amylase and lipase levels in patients using glucagonlike peptide-1 receptor agonists or dipeptidyl-peptidase-4 inhibitors in the outpatient setting.** *Endocr Pract.* 2012; **18**(4): 472–477.
[PubMed Abstract](#) | [Publisher Full Text](#)
30. Busch SJ, Hoffmann P, Sahota P, *et al.*: **Studies in rodents with the dipeptidyl peptidase-4 inhibitor vildagliptin to evaluate possible drug-induced pancreatic histological changes that are predictive of pancreatitis and cancer development in man.** *Diabetes Obes Metab.* 2013; **15**(1): 72–76.
[PubMed Abstract](#) | [Publisher Full Text](#)
31. Mizukami H, Inaba W, Takahashi K, *et al.*: **The effects of dipeptidyl-peptidase-IV inhibitor, vildagliptin, on the exocrine pancreas in spontaneously diabetic Goto-Kakizaki rats.** *Pancreas.* 2013; **42**(5): 786–794.
[PubMed Abstract](#) | [Publisher Full Text](#)
32. Huang KF, Liaw SS, Huang WL, *et al.*: **Structures of human Golgi-resident glutaminyl cyclase and its complexes with inhibitors reveal a large loop movement upon inhibitor binding.** *J Biol Chem.* 2011; **286**(14): 12439–12449.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Gherardini PF, Wass MN, Helmer-Citterich M: **Convergent evolution of enzyme active sites is not a rare phenomenon.** *J Mol Biol.* 2007; **372**(3): 817–845.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. Konagurthu AS, Whisstock JC, Stuckey PJ, *et al.*: **MUSTANG: a multiple structural alignment algorithm.** *Proteins.* 2006; **64**(3): 559–574.
[PubMed Abstract](#) | [Publisher Full Text](#)
35. Chakraborty S: **An automated flow for directed evolution based on detection of promiscuous scaffolds using spatial and electrostatic properties of catalytic residues.** *PLoS One.* 2012; **7**(7): e40408.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Ahyauch H, Villar AV, Alonso A, *et al.*: **Modulation of PI-specific phospholipase C by membrane curvature and molecular order.** *Biochemistry.* 2005; **44**(34): 11592–11600.
[PubMed Abstract](#) | [Publisher Full Text](#)
37. Villar AV, Alonso A, Goni FM: **Leaky vesicle fusion induced by phosphatidylinositol-specific phospholipase C: observation of mixing of vesicular inner monolayers.** *Biochemistry.* 2000; **39**(46): 14012–14018.
[PubMed Abstract](#) | [Publisher Full Text](#)
38. Nabeno M, Akahoshi F, Kishida H, *et al.*: **A comparative study of the binding modes of recently launched dipeptidyl peptidase IV inhibitors in the active site.** *Biochem Biophys Res Commun.* 2013; **434**(2): 191–196.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Heinz DW, Ryan M, Bullock TL, *et al.*: **Crystal structure of the phosphatidylinositolspecific phospholipase C from *Bacillus cereus* in complex with myo-inositol.** *EMBO J.* 1995; **14**(16): 3855–3863.
[PubMed Abstract](#) | [Free Full Text](#)
40. Chakraborty S: **DOCLASP - Docking ligands to target proteins using spatial and electrostatic congruence extracted from a known holoenzyme, and applying simple geometrical transformations [v1; ref status: awaiting peer review, <http://f1000r.es/48g>].** *F1000Res.* 2014; **3**: 262.
[Publisher Full Text](#)
41. Abbott CA, McCaughan GW, Gorrell MD: **Two highly conserved glutamic acid**

- residues in the predicted beta propeller domain of dipeptidyl peptidase IV are required for its enzyme activity. *FEBS Lett.* 1999; **458**(3): 278–284.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Butler PC, Dry S, Elashoff R: **Glip-1-based therapy for diabetes: what you do not know can hurt you.** *Diabetes Care.* 2010; **33**(2): 453–455.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. Butler PC, Elashoff M, Elashoff R, *et al.*: **A critical analysis of the clinical use of incretin-based therapies: Are the GLP-1 therapies safe?** *Diabetes Care.* 2013; **36**(7): 2118–2125.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 44. Parks M, Rosebraugh C: **Weighing risks and benefits of liraglutide—the FDA's review of a new antidiabetic therapy.** *N Engl J Med.* 2010; **362**(9): 774–777.
[PubMed Abstract](#) | [Publisher Full Text](#)
 45. Garg R, Chen W, Pendergrass M: **Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis.** *Diabetes Care.* 2010; **33**(11): 2349–2354.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 46. Jura N, Archer H, Bar-Sagi D: **Chronic pancreatitis, pancreatic adenocarcinoma and the black box in-between.** *Cell Res.* 2005; **15**(1): 72–77.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. Engel SS, Round E, Golm GT, *et al.*: **Safety and tolerability of sitagliptin in type 2 diabetes: pooled analysis of 25 clinical studies.** *Diabetes Ther.* 2013; **4**(1): 119–145.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 48. Nyborg NC, Molck AM, Madsen LW, *et al.*: **The human GLP-1 analog liraglutide and the pancreas: evidence for the absence of structural pancreatic changes in three species.** *Diabetes.* 2012; **61**(5): 1243–1249.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 49. Tatarkiewicz K, Belanger P, Gu G, *et al.*: **No evidence of drug-induced pancreatitis in rats treated with exenatide for 13 weeks.** *Diabetes Obes Metab.* 2013; **15**(5): 417–426.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 50. Vrang N, Jelsing J, Simonsen L, *et al.*: **The effects of 13 wk of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF rats: a quantitative and qualitative analysis revealing no evidence of drug-induced pancreatitis.** *Am J Physiol Endocrinol Metab.* 2012; **303**(2): E253–E264.
[PubMed Abstract](#) | [Publisher Full Text](#)
 51. Bjerre Knudsen L, Madsen LW, Andersen S, *et al.*: **Glucagon-like Peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation.** *Endocrinology.* 2010; **151**(4): 1473–1486.
[PubMed Abstract](#) | [Publisher Full Text](#)
 52. Hegedus L, Moses AC, Zdravkovic M, *et al.*: **GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide.** *J Clin Endocrinol Metab.* 2011; **96**(3): 853–860.
[PubMed Abstract](#) | [Publisher Full Text](#)
 53. Capuano A, Sportiello L, Maiorino MI, *et al.*: **Dipeptidyl peptidase-4 inhibitors in type 2 diabetes therapy—focus on alogliptin.** *Drug Des Devel Ther.* 2013; **7**: 989–1001.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 54. Gupta V, Kalra S: **Choosing a gliptin.** *Indian J Endocrinol Metab.* 2011; **15**(4): 298–308.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 55. Bachovchin DA, Koblan LW, Wu W, *et al.*: **A high-throughput, multiplexed assay for superfamily-wide profiling of enzyme activity.** *Nat Chem Biol.* 2014; **10**(8): 656–663.
[PubMed Abstract](#) | [Publisher Full Text](#)
 56. Scheen A: **Gliptins (dipeptidyl peptidase-4 inhibitors) and risk of acute pancreatitis.** *Expert Opin Drug Saf.* 2013; **12**(4): 545–557.
[PubMed Abstract](#) | [Publisher Full Text](#)
 57. Kaufman MB: **Drug-induced pancreatitis: A Potentially Serious and Underreported Problem.** *PT.* 2013; **38**(6): 349–351.
[PubMed Abstract](#) | [Free Full Text](#)
 58. Bernstein FC, Koetzle TF, Williams GJ, *et al.*: **The Protein Data Bank: a computer-based archival file for macromolecular structures.** *J Mol Biol.* 1977; **112**(3): 535–542.
[PubMed Abstract](#) | [Publisher Full Text](#)
 59. Baker NA, Sept D, Joseph S, *et al.*: **Electrostatics of nanosystems: application to microtubules and the ribosome.** *Proc Natl Acad Sci U S A.* 2001; **98**(18): 10037–10041.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 60. Dolinsky TJ, Nielsen JE, McCammon JA, *et al.*: **PDB2PQR: an automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations.** *Nucleic Acids Res.* 2004; **32**(suppl 2): W665–W667.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Current Referee Status:



Version 2

Referee Report 20 January 2015

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Mark D Gorrell

Molecular Hepatology, Centenary Institute, Newtown, NSW, Australia

Thank you to the authors for developing this paper.

I have some further comments.

1. The primary issue now is the speculation in the title.

The title seeks to extrapolate the obtained data on two compounds to suggest that it is applicable to all DPP-IV inhibitors.

That is, the speculation of this paper is that the presented data is relevant to an entire drug class. The comments and the title should be restricted to one or two of the compounds that were studied in this paper. Moreover, K-579 is not a diabetes drug. In this context, the title needs changing to avoid ambiguity.

I suggest:

"The dipeptidyl peptidase IV inhibitor vildagliptin used in type 2 diabetes inhibits a phospholipase C: a case of promiscuous scaffolds in proteins."

or

"The dipeptidyl peptidase IV inhibitors vildagliptin and K-579 inhibit a phospholipase C: a case of promiscuous scaffolds in proteins."

2. This study complements the much broader work using focused, direct technology for measuring and detecting off-target inhibition. That paper is published in Nature Chemical Biology in 2014 ([Bachovchin et al. 2014](#)). That study similarly showed that vildagliptin inhibits enzymes other than DPP-IV. That study showed that DPP4 inhibitors differ, such that sitagliptin does not inhibit other enzymes.

The authors need to comment and restrict their conclusions to the compounds that they studied rather than imply that DPP-IV inhibiting compounds that they did not study, such as sitagliptin, have similar characteristics to the compounds that they did study.

3. The data of this study is biochemical yet 16 of the cited references concern the safety of DPP-IV inhibition. The manuscript now carefully does not draw a link to drug safety; the title needs to do the same.
4. As the paper is focused upon DPP-IV structure and function, more papers on this topic could be cited and linked with the data. For example, the author's amendment mentions contacts in DPP-IV at Glu205, Glu206 and Tyr662. The authors could state that Glu205 and Glu206 have been shown to be essential for catalysis by DPP-IV and cite the paper [Abbott *et al.* \(1999\)](#).

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: This reviewer recently received a speaker honorarium from Boehringer Ingelheim, which manufactures linagliptin.

Author Response 28 Jan 2015

Sandeep Chakraborty, Tata Institute of Fundamental Research, India

We would like to thank you for your positive comments, and your informative suggestions.

We agree with your suggested change in the title. In the latest version, we have also cited the research you have brought to our attention.

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 26 March 2014

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Mark D Gorrell

Molecular Hepatology, Centenary Institute, Newtown, NSW, Australia

The successful targeting of DPP4 using small molecule compounds to treat type 2 diabetes has attracted a great deal of attention towards the study of this protease.

The authors applied sophisticated techniques that they have developed in order to discover that two DPP4 inhibitors, including one that is in limited clinical use, can to some extent inhibit the activity of a bacterial lipase (PI-PLC). Many lipases and esterases and hydrolases including DPP4 and related enzymes use the *alpha/beta* hydrolase fold and the authors show how this related protein topology can place the residues in positions that are sufficiently similar to interact with an inhibitor.

The major difficulty with this paper is that it attempts to connect these data with possible clinical outcomes. No evidence for such a link is presented. Therefore, the title and much of the conclusions need to be modified so that they reflect the data without speculation.

Two inhibitors of DPP4, LAF237 and K-579, were studied. K-579 is not in clinical use. LAF237 is licensed in Europe and is known to exhibit some inhibition of the DPP4-related proteases DPP8 and DPP9. The extent of inhibition of DPP8 and DPP9 by LAF237 is believed not to have physiological effects in humans. The IC₅₀ of LAF237 on DPP9 is less than 0.01 mM. The IC₅₀ of LAF237 on bacterial PI-PLC is 0.1mM, which is close to the lower limit of detection of inhibition of an enzyme. No mammalian homolog of PI-PLC was examined.

The literature that the authors cite to suggest that DPP4 inhibition might be detrimental for human health, particularly the pancreas, is data on sitagliptin or exenatide. Exenatide is not a DPP4 inhibitor and sitagliptin is quite different to LAF237, both in protease specificity and in chemical structure. The contact points of LAF237 and sitagliptin in the catalytic site of DPP4 differ considerably. The authors present no data on sitagliptin or any other DPP4 inhibitor (other than LAF237) that is in the clinic.

The images of overlaid catalytic triads of various enzymes presented in Fig 1 and Fig 3 need to be depicted in 3D in order to evaluate how close they are in 3D. Intermolecular distances should be shown on these figures. To convince the reader that LAF237 sits into and makes contacts with enzymes other than DPP4, we need to see the compound docked into the structure of each enzyme of interest.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 10 Dec 2014

Sandeep Chakraborty, Tata Institute of Fundamental Research, India

We would like to thank you for taking the time to review this paper, and also for your insightful comments. We also apologize for the inordinate time taken to respond to your comments. A lot of this time was spent in understanding docking methods, instead of blindly applying this to the problem at hand. A by-product of this learning process was the implementation of a new method (DOCLASP) for docking molecules to proteins¹. We have docked vildagliptin to the PI-PLC structure complexed with myo-inositol using DOCLASP. Based on your suggestion, we have also done a comprehensive analysis of all 76 known DPP4 structures liganded to inhibitors till date.

Please find out detailed responses to your comments below.

- *The successful targeting of DPP4 using small molecule compounds to treat type 2 diabetes has attracted a great deal of attention towards the study of this protease. The authors applied sophisticated techniques that they have developed in order to discover that two DPP4 inhibitors, including one that is in limited clinical use, can to some extent inhibit the activity of a bacterial lipase (PI-PLC). Many lipases and esterases and hydrolases including DPP4 and related enzymes use the alpha/beta hydrolase fold and the authors show how this related protein topology can place the residues in positions that are sufficiently similar to interact with an inhibitor. The major difficulty with this paper is that it attempts to connect these data with possible clinical outcomes. No evidence for such a link is presented. Therefore, the title and much of the conclusions need to be modified so that they reflect the data without speculation.*

We have tried to keep away from taking sides on the clinical outcomes, since that is not our forte. Also, we believe our title is innocuous in that context - it just speaks of promiscuous scaffolds. We only highlight that if (and only if) our data of PIPLC inhibition holds true for human lipases, then it might provide some arguing points for those worried about the side effects of these drugs.

For example, we say 'The reported elevated levels of serum lipase, although contested, could be rationalized by inhibition of lipases reported here'. If you could kindly point out specifically any speculations that is unwarranted, we will modify those.

- *Two inhibitors of DPP4, LAF237 and K-579, were studied. K-579 is not in clinical use. LAF237 is licensed in Europe and is known to exhibit some inhibition of the DPP4-related proteases DPP8 and DPP9. The extent of inhibition of DPP8 and DPP9 by LAF237 is believed not to have physiological effects in humans.*

Since this study does not emphasize on the clinical relevance of the inhibitions (but on the methodology of finding such interactions), and we are not a group specializing in diabetes, we believe the choice of the inhibitors would not alter our reasoning our conclusions.

- *The IC50 of LAF237 on DPP9 is less than 0.01 mM. The IC50 of LAF237 on bacterial PI-PLC is 0.1mM, which is close to the lower limit of detection of inhibition of an enzyme.*

We agree to this point. However, K-579 was inhibiting even at nanomolar concentrations.

- *No mammalian homolog of PI-PLC was examined.*

We are currently evaluating that possibility.

- *The literature that the authors cite to suggest that DPP4 inhibition might be detrimental for human health, particularly the pancreas, is data on sitagliptin or exenatide. Exenatide is not a DPP4 inhibitor and sitagliptin is quite different to LAF237, both in protease specificity and in chemical structure.*

We were referring to the inhibitor part of the data, but that point needs to be made explicit as you have correctly pointed out. Also, we agree that the possible difference of sitagliptin with LAF237 needs to be stated. We have modified the text to include these criticisms. Once again, we reiterate we intend not to comment on clinical outcomes or debates, but to suggest a rational methodology to act as a guide for tests that look for possible interactions.

- *The contact points of LAF237 and sitagliptin in the catalytic site of DPP4 differ considerably. The authors present no data on sitagliptin or any other DPP4 inhibitor (other than LAF237) that in is the clinic.*

We have included a comprehensive study on the contact points of various inhibitors. Once again, this does not negate any of our conclusions.

- *The images of overlaid catalytic triads of various enzymes presented in Fig 1 and Fig 3 need to be depicted in 3D in order to evaluate how close they are in 3D.*

The 3D images of the superimposition of these enzymes are not pleasing to the eye, since they lack structural homology. However, we have added a PyMol script in case someone wishes to do that (Superimposeproteins.p1m). The script specifies the color coding of the residues.

- *Intermolecular distances should be shown on thee figures.*

Once again, we think that the intermolecular distances clutter the figure. The superimposition gives an approximate idea of the congruence. The exact values are specified in Table 1. We have modified the legend of Fig.3 to specify that.

- *To convince the reader that LAF237 sits into and makes contacts with enzymes other than DPP4, we need to see the compound docked into the structure of each enzyme of interest.*

As mentioned previously, we have docked sitagliptin to PI-PLC using DOCLASP¹. We have provided the Pymol script as supplementary data to help visualize the docking. There is no solved structure where LAF237 inhibits DPP4.

Once again, we are thankful for the comments. We hope that we have addressed your concerns by the changes that we have made, and that the manuscript will be found suitable in the modified form.

References

1. Chakraborty S. *DOCLASP* - Docking ligands to target proteins using spatial and electrostatic congruence extracted from a known holoenzyme and applying simple geometrical transformations [v2; ref status: awaiting peer review, <http://f1000r.es/4pb>] *F1000Research* 2014, **3**:262 (doi: [10.12688/f1000research.5145.2](https://doi.org/10.12688/f1000research.5145.2))

Competing Interests: No competing interests were disclosed.

Referee Report 06 March 2014

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Rodney Rouse

Division of Applied Regulatory Science, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, USA

Disclaimer: I lack the protein chemistry expertise to comment on the assumptions and protein chemistry used in the computational method described in this article.

The title and abstract are appropriate. The overall experimental design is simple but strong and well suited for this project. The methods were generally well described. The conclusions are not overstated and any implications are justified based on the presented data. The article is very well written.

This is a very interesting study that uses a previously defined computational method, Catalytic Active Site Prediction (CLASP), that compares structural and charge similarities of catalytic sites to identify

functionally similar proteins. This methodology was used to assess the potential for adverse events based on off target effects of the inhibitors of DPP-IV. Using CLASP, the authors had previously identified a *Bacillus cerus* phosphoinositide specific phospholipase-C (PI-PLC) as similar in active catalytic site to the enzyme, DPP-IV. They used laboratory techniques to verify this finding.

In the present study, the authors demonstrated the ability of two separate DPP-IV inhibitors to significantly reduce the activity of this PI-PLC in the lab. Subsequent to this experimentation, the authors returned CLASP to identify catalytic sites in other proteins that might also be inhibited by DPP-IV inhibitors thereby yielding unforeseen inhibition and biological effects. As applied to the case of DPP-IV inhibitors, which are not extremely specific, the authors identify a number of other proteins that could be promiscuously impacted by DPP-IV inhibitors thereby providing mechanisms for unexpected adverse events. Although the significance of DPP-IV inhibitor related adverse events has yet to be determined, the fact that changes have been reported non-clinically and clinically are undeniable. Eventually, the benefit of these molecules may far outweigh their associated risks, but the authors provide a potential path forward for investigation of unexpected events with this class of drug. If contradictory reports persist, this path may require further illumination.

The approach is theoretically similar to using structural similarities to identify off target receptor binding and consequent biological effects, an expanding approach in safety assessment and in identification of mechanisms for adverse events in the pharmaceutical lifecycle. Similarly, this method could be predictive for off target effects and suggest what those effects might be. However, whether this is a method that can be generally applicable to other molecules is beyond my ability to comment and the scope of this work.

Comments/Suggestions:

1) Were the inhibition experiments done in duplicate, triplicate, etc? Some slight expansion of the protocols would help with attempts to replicate.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Author Response 10 Mar 2014

Adela Rendón-Ramírez, Unidad de Biofísica CSIC UPV, Spain

Dear Dr Rouse,

We would like to thank you for taking the time and reviewing our paper. Your positive comments encourage us to further our research in this area.

We concur with your statement - *"Eventually, the benefit of these molecules may far outweigh their associated risks"*. And it is our endeavor to improve the accuracy and generality of our method through different compounds. We would specifically like to highlight another case of antagonist binding identified through CLASP, although in this case most alkaline phosphatases were not affected - [Chakraborty et al.\(2012\)](#)

The data for PI-PLC inhibition using DPP4 inhibitors, as shown in Figure 2, are average values of two closely similar experiments. We will revise the manuscript to include this point when we hear

from another referee.

Best regards,

Sandeep Chakraborty and Adela Rendón-Ramirez

Competing Interests: No competing interests were disclosed.
